

the art. This antibody can be labeled with the radioactive isotope ¹²⁵I, for example, by methods routinely practiced in the art. In a heterogeneous assay, the GST-GLUTX fusion protein can be anchored to glutathione-agarose beads. The
5 interactive binding partner can then be added in the presence or absence of the test compound in a manner that allows interaction and binding to occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal antibody can be added to the system
10 and allowed to bind to the complexed components. The interaction between GLUTX and the interactive binding partner can be detected by measuring the amount of radioactivity that remains associated with the glutathione-agarose beads. A successful inhibition of the interaction
15 by the test compound will result in a decrease in measured radioactivity.

Alternatively, the GST-GLUTX fusion protein and the interactive binding partner can be mixed together in liquid in the absence of the solid glutathione-agarose beads. The
20 test compound can be added either during or after the species are allowed to interact. This mixture can then be added to the glutathione-agarose beads and unbound material is washed away. Again the extent of inhibition of the GLUTX/binding partner interaction can be detected by adding
25 the labeled antibody and measuring the radioactivity associated with the beads.

In another embodiment of the invention, these same techniques can be employed using peptide fragments that correspond to the binding domains of GLUTX and/or the
30 interactive or binding partner (in cases where the binding partner is a protein), in place of one or both of the full length proteins. Any number of methods routinely practiced in the art can be used to identify and isolate the binding

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sites. These methods include, but are not limited to, mutagenesis of the gene encoding one of the proteins and screening for disruption of binding in a co-immunoprecipitation assay. Compensating mutations in the
5 gene encoding the second species in the complex can then be selected. Sequence analysis of the genes encoding the respective proteins will reveal the mutations that correspond to the region of the protein involved in interactive binding. Alternatively, one protein can be
10 anchored to a solid surface using methods described above, and allowed to interact with and bind to its labeled binding partner, which has been treated with a proteolytic enzyme, such as trypsin. After washing, a short, labeled peptide comprising the binding domain may remain associated with the
15 solid material, which can be isolated and identified by amino acid sequencing. Also, once the gene coding for the intracellular binding partner is obtained, short gene segments can be engineered to express peptide fragments of the protein, which can then be tested for binding activity
20 and purified or synthesized.

XV. Methods for Reducing GLUTX Expression

Expression of GLUTX can be reduced through the use of modulatory compounds identified through the use of the
25 screening methods described above. In addition, endogenous GLUTX gene expression can also be reduced by inactivating or "knocking out" the GLUTX gene or its promoter using targeted homologous recombination (see, for example, U.S. Patent No. 5,464,764). For example, a mutant, non-functional GLUTX
30 (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous GLUTX gene (either the coding regions or regulatory regions of the GLUTX gene) can be used, with or without a selectable marker and/or a negative

selectable marker, to transfect cells that express GLUTX-3
in vivo. Insertion of the DNA construct, via targeted
homologous recombination, results in inactivation of the
GLUTX gene. Such approaches are particularly suited for use
5 in developing animal models to study the role of GLUTX; in
this instance, modifications to ES (embryonic stem) cells
can be used to generate animal offspring with an inactive
GLUTX gene. However, a knock out approach can be adapted
for use in humans, provided the recombinant DNA constructs
10 are directly administered or targeted to the required site
in vivo using appropriate viral vectors.

Alternatively, endogenous GLUTX gene expression can
be reduced by targeting deoxyribonucleotide sequences
complementary to the regulatory region of the GLUTX gene
15 (i.e., the GLUTX promoter and/or enhancers) to form triple
helical structures that prevent transcription of the GLUTX
gene in target cells in the body (Helene, *Anticancer Drug*
Res. 6:569, 1981; Helene et al., *Ann. N.Y. Acad. Sci.*
660:27, 1992; and Maher, *Bioassays* 14:807, 1992).

20 In addition, as discussed above, anti-sense
molecules, ribozymes, and peptide nucleic acids can be used
to reduce GLUTX expression.

XVI. Assays for the Identification of Compounds that
25 Ameliorate Disorders Associated with Aberrant GLUTX
Expression or Activity

Compounds, including, but not limited to, compounds
identified via assay techniques such as those described
above may be useful for the treatment of disorders
30 associated with aberrant GLUTX expression or aberrant GLUTX
activity.

While animal model-based assays are particularly
useful for the identification of such therapeutic compounds,